Product Manual

Human Adiponectin ELISA Kit

Catalog Numbers

MET- 5052	96 assays
MET- 5052- 5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Adiponectin is an important protein hormone with a variety of paracrine and endocrine effects on metabolism and inflammation within the cardiovascular system. Also known as adipocyte complement related protein of 30 kDa (Acrp30), adiponectin's pleiotropic actions influence macrophages, endothelial cells (ECs), endothelial progenitor cells (EPCs), vascular smooth muscle cells (VSMCs), leukocytes, and cardiomyocytes. Among its many functions include its involvement with fatty acid catabolism, type 2 diabetes, atherogenesis, adipocyte differentiation, and it is inversely related to obesity. Adiponectin is secreted almost exclusively by adipocytes into the bloodstream and is abundant in plasma compared to other hormones. Adiponectin molecules self-associate to form 70 kDa non-covalently bound homotrimers, which can associate further into hexamers or dodecamers. High levels of adiponectin are associated with a reduced risk of heart attack while low levels are associated with obesity and adipose tissue dysfunction. Visceral fat accumulation has been shown to be associated with lower adiponectin levels than subcutaneous fat. Adiponectin is an excellent biological marker for understanding adipocyte and cardiovascular disorders.

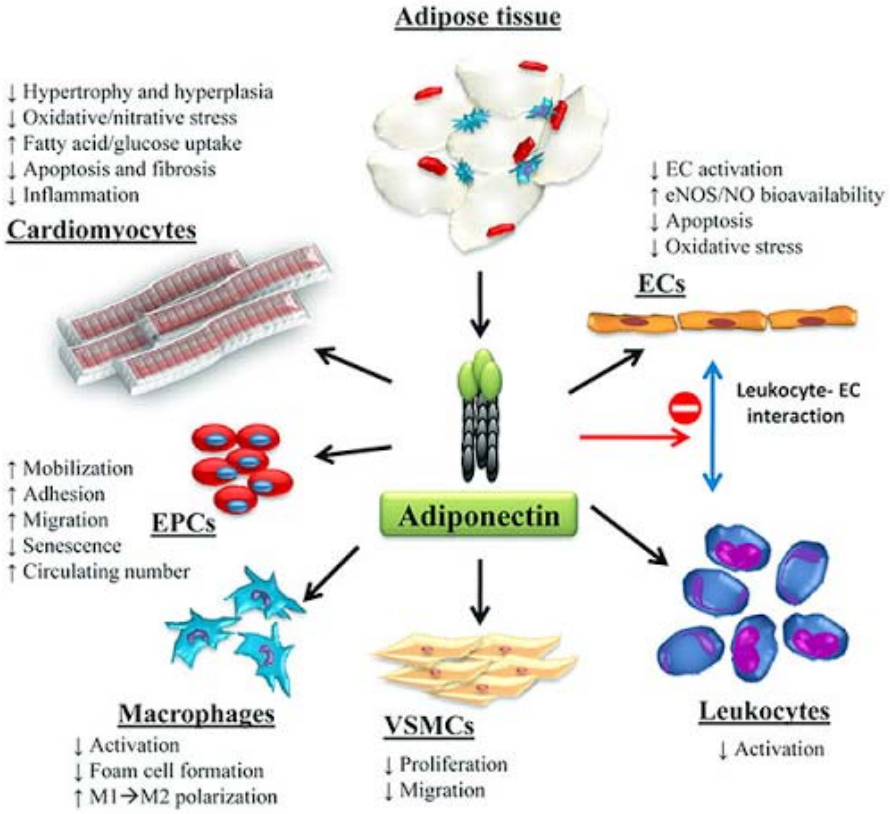

Figure 1: Adiponectin's Pathways

Cell Biolabs' Human Adiponectin ELISA Kit is an enzyme immunoassay developed for detection and quantitation of human adiponectin. The kit utilizes a native human adiponectin standard and has a detection sensitivity limit of 30 pg/mL. Each kit provides sufficient reagents to perform up to 96 assays including the standard curve and samples.

Assay Principle

This assay is based on a sandwich ELISA format. Adiponectin present in samples or standards bind to the anti-adiponectin antibodies pre-adsorbed on the microtiter plate. Next, a biotinylated anti-adiponectin antibody is added to the plate well and binds to the captured adiponectin. A streptavidin-enzyme conjugate is then added, which binds to the biotin of the second antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of adiponectin present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified native human adiponectin. Sample concentration is then determined by comparing to the known values of the standard curve.

Related Products

1. STA-384: Total Cholesterol Assay Kit (Colorimetric)
2. STA-390: Total Cholesterol Assay Kit (Fluorometric)
3. STA-396: Serum Triglyceride Quantitation Kit (Colorimetric)
4. STA-618: Free Fatty Acid Assay Kit (Colorimetric)
5. STA-680: Glucose Assay Kit (Colorimetric)
6. MET-5014: NAD^+/NADH Assay Kit (Colorimetric)
7. MET-5018: $NADP^+$/NADPH Assay Kit (Colorimetric)
8. MET-5030: NAD^+/NADH Assay Kit (Fluorometric)
9. MET-5031: $NADP^+$/NADPH Assay Kit (Fluorometric)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Adiponectin Antibody Coated Plate (Part No. 50521B): One strip well 96-well plate
2. Anti-Adiponectin Biotinylated Antibody (1000X) (Part No. 50522D): One 10 µL vial of anti-adiponectin antibody
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL tube
4. Assay Diluent (Part No. 310804): One 50 mL bottle
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle
7. Stop Solution (Part. No. 310808): One 12 mL bottle

Box 2 (shipped on blue ice packs)

1. Adiponectin Standard (Part No. 50523D): One 15 µL vial of 10 µg/mL human adiponectin

Materials Not Supplied

1. Adiponectin samples: human serum, plasma, lysates
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store Adiponectin Standard at -80ºC and avoid freeze/thaw. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Adiponectin Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Adiponectin Antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.
- Substrate Solution: Prior to use, warm the Substrate Solution to room temperature.

Preparation of Samples

Samples should be assayed immediately or stored at -80ºC prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples.

- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Normal plasma samples require about 1,000 to 10,000 fold dilution with 1X PBS containing 0.1% BSA immediately before running the ELISA.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Assay immediately or store samples at -80ºC for up to three months. Normal serum samples require about 1,000 to 10,000 fold dilution with 1X PBS containing 0.1% BSA immediately before running the ELISA.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in 1X PBS containing 0.1% BSA as needed.

- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in 1X PBS containing 0.1% BSA as needed.

Preparation of Standard Curve

1. Prepare fresh standards by diluting the Adiponectin Standard from 10 µg/mL to 10 ng/mL (1:1000) in Assay Diluent (Example: Add 5 µL of Adiponectin Standard stock tube to 4.995 mL of Assay Diluent).
2. Prepare a series of the remaining adiponectin standards in the concentration range of 2 ng/mL – 0.015 ng/mL by diluting the 10 ng/mL according to Table 1 below.

Standard Tubes	10 ng/mL Human Adiponectin Standard (µL)	Assay Diluent (µL)	Adiponectin (ng/mL)
1	200	800	2
2	500 of Tube #1	500	1
3	500 of Tube #2	500	0.5
4	500 of Tube #3	500	0.25
5	500 of Tube #4	500	0.125
6	500 of Tube #5	500	0.063
7	500 of Tube #6	500	0.031
8	0	500	0

Table 1. Preparation of Adiponectin Standard Curve.

Note: Do not store diluted adiponectin standard solutions.

Assay Protocol

Note: Each Adiponectin Standard and unknown samples should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 100 µL of adiponectin standards or samples to the Anti-Adiponectin Antibody Coated Plate. Each sample, standard, blank, and control should be assayed in duplicate.
2. Incubate 1 hour at room temperature on an orbital shaker.
3. Remove the solution from the wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Biotinylated Anti-Adiponectin Antibody to each well.
5. Incubate 1 hour at room temperature on an orbital shaker.
6. Remove the solution from the wells. Wash the strip wells 5 times according to step 3 above.

7. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
8. Incubate 1 hour at room temperature on an orbital shaker.
9. Remove the solution from the wells. Wash the strip wells 5 times according to step 3 above.
10. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
11. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
12. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Human Adiponectin ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

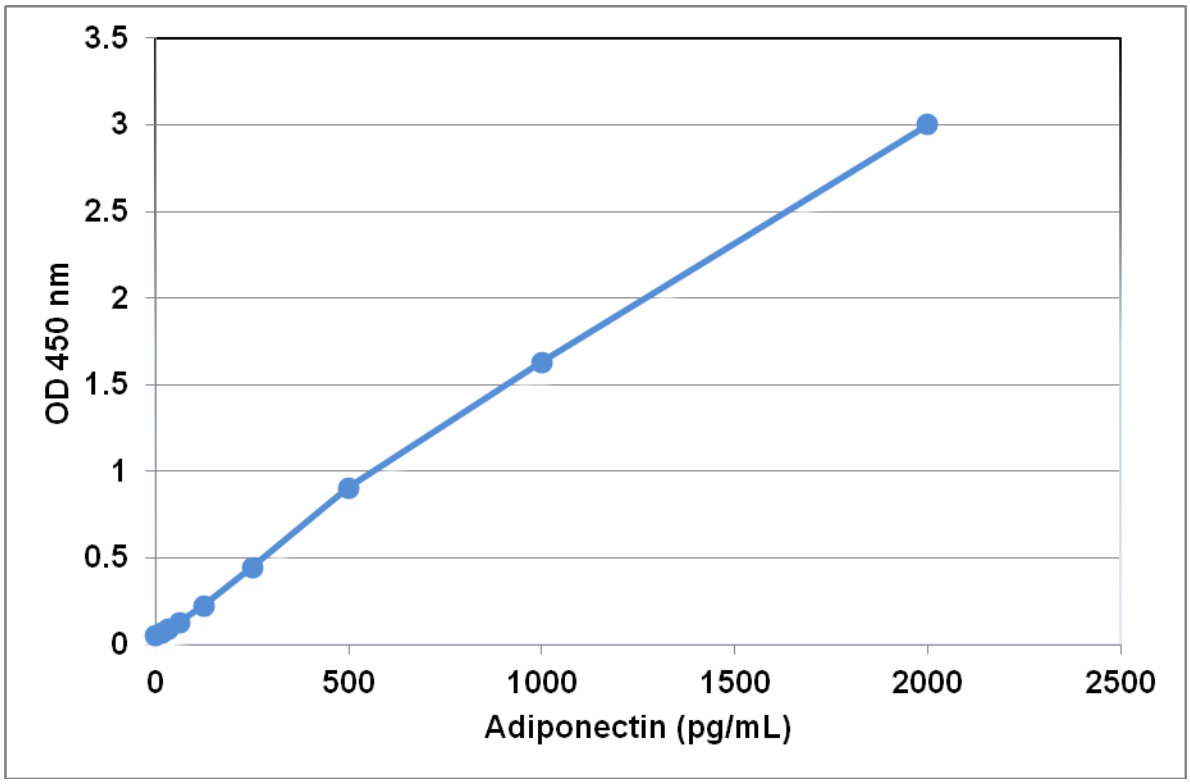

Figure 2: Human Adiponectin ELISA Standard Curve

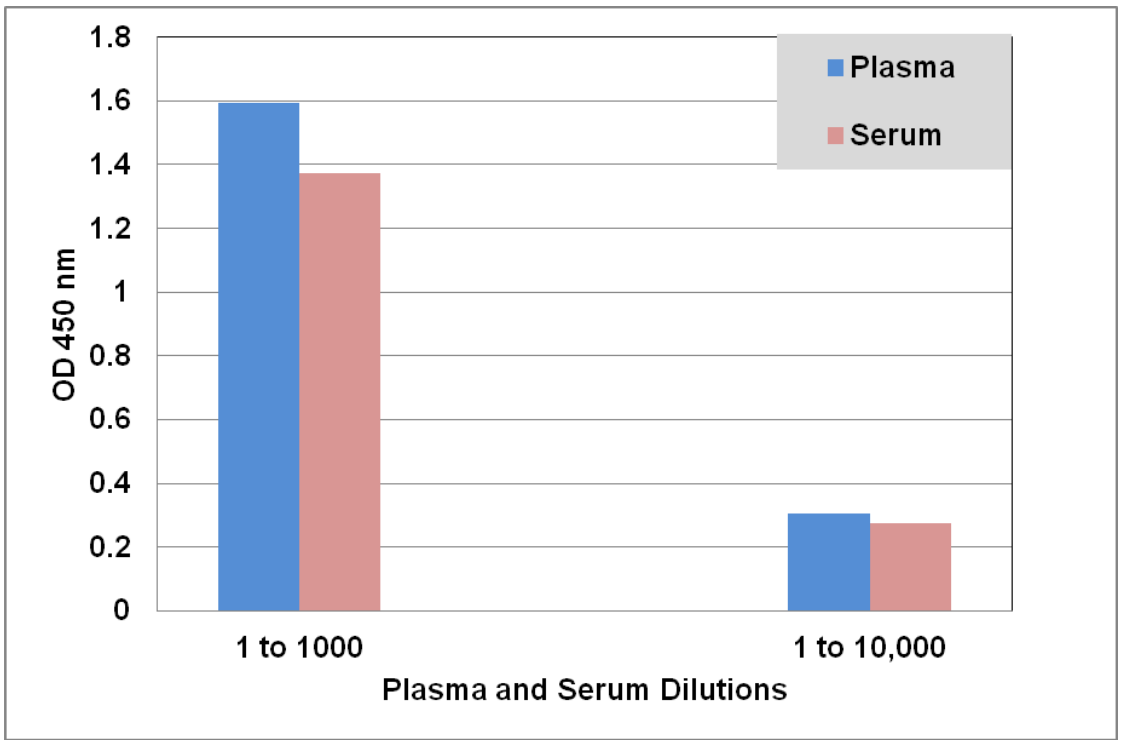

Figure 3: Adiponectin Levels in Human Plasma and Serum. Human Plasma and Serum were diluted 1:1000 and 1:10,000 in Assay Diluent and tested according to the product insert.

References

1. Arita, Yukio, et al. (1999) *Biochemical and biophysical research communications* **257.1**: 79-83.
2. Hotta, Kikuko, et al. (2000) *Arteriosclerosis, thrombosis, and vascular biology* **20.6**: 1595-99.
3. Yamauchi, Toshimasa, et al. (2001) *Nature medicine* **7.8**: 941-46.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com